REMARKS

Claim 1 has been amended by merging claim 62 into claim 1 to recite that "sizing the non-pegylated liposomes formed to about 0.06 μm to form a liposomal composition" and "removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution." Claim 1 has also been amended to recite mixtures as opposed to derivatives. Support for this claim amendment can be found in paragraph [0016] of the published patent application. Claim 8 has been corrected to require that the molar ratio of phospholipid is "about 1:0.7." Support for this amendment can be found at page 5, lines 7 – 8. No new matter has been added by the claim amendments. Claim 62 is currently canceled. Claims 1-8, 10, 12, and 14 – 22 remain pending.

ARGUMENTS

I. Claim Rejections – 35 U.S.C. § 103(a) – generally

Applicants remind the Office that the Supreme Court case KSR Int'l Co. v. Teleflex Inc., 127 S. Ct. 1727 (2007) addressed the sufficiency of a reference combination and indicated that, for a combination rejection, there must be an important reason for combination of the references and there must be a reasonable expectation of success in producing the claimed invention. The Supreme Court noted in KSR that:

> [A] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art. Although common sense directs one to look with care at a patent application that claims as innovation the combination of two known devices according to their established functions, it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does.

KSR, 127 S. Ct. at 1740-41. Applicants submit and will demonstrate herein below that arguments presented in the Office Action have failed to provide a sufficient reason, sufficient motivation and a reasonable expectation of success in the combination of the references. In fact, applicants will show that there is just the opposite – there is no motivation to combine these references and as such The Office Action has used improper hindsight has been used to provide motivation to combine the reference. In addition, and notably, the combination of all of the references do not teach each and every element of the presently amended claims.

Applicants respectfully point out that the references have been misconstrued. Certain elements from some references have been combined with elements from other references with improper or in some cases, no consideration of how the elements were used in the references to argue that the present invention is obvious. Applicants show herein below that one skilled in the art would not be motivated to make such a combination when reading the references in context for what they teach. In many instances the cited references teach completely different and almost opposite techniques (i.e. liposome loading as opposed to liposome stability, for example), so it would not make any rational sense to combine bits and pieces from each reference to arrive at the presently claimed invention.

II. Claim Rejections – 35 U.S.C. § 103(a) – Kirpotin/Papahadjopoulos

In paragraph 4 on page 2, claims 1-8, 10, 12, 14-22 and 62 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin (US patent 6,110,491) in view of Papahadjopoulos (US patent 4,235,871). Applicants submit that the two cited references, alone or in combination fail to teach or suggest each and every element of the presently amended claims. Each of these references is discussed below.

A. Kirpotin does not teach or suggest each and every element of the claimed invention.

1. Kirpotin teaches away from using ammonium sulfate in a hydration buffer.

The Office Action states that:

> Kirpotin discloses a method of preparation of liposomes forming lipid film and hydrating it with a buffer containing ammonium sulphate. (Example 7). Kirpotin also teaches that if necessary, to achieve an osmolarity of 377 mmole/kg, sucrose could be added to the medium. (Example 8) The liposomes contain hydrogenated egg phospholipid and cholesterol. Doxorubicin is loaded into the preformed liposomes (Example 7). Although in the examples Kirpotin uses PEG-phospholipids, on col. 9 lines 22-23 teaches either the naturally occurring or synthetic phospholipids which implies that use of PEG phospholipids for the method of preparations is not necessary. What is lacking in Kirpotin is the teaching of the amount of aqueous medium added to per mol of phospholipid.

First, applicants respectfully note that Kirpotin is concerned with liposome loading and does not teach anything about a process for making a long-circulating non-pegylated liposome.

Second, Kirpotin's example 7 teaches that loading of the liposomes "containing ammonium sulfate in the absence of ammonium ion gradient absorbed very small amounts of doxorubicin." See col. 14, lines 63-65. Further, Kirpotin states: "while the liposomes containing ammonium polyacrylate absorbed considerable amounts of doxorubicin even without ammonium ion gradient." See col. 14, lines 65-67. Thus, this example is teaching away from using ammonium sulfate in the loading buffer as ammonium polyacrylate provided a much better loading efficiency. The references teaches that ammonium polyacrylate works much better than ammonium sulphate in liposome loading. Further, Kirpotin's Example 7 uses ammonium sulphate inside the liposomes via the hydration medium but it says: "[a]t this temperature, doxorubicin did not form a detectable precipitate in the presence of sulfate anion, but was visibly precipitated by a polyacrylate anion," which again teaches away from the use of ammonium sulphate. See col. 14, 40-42.

Example 7 and 8 thus show that the use of ammonium sulphate in the hydrating medium in a pegylated (PEG-DSPE containing) liposome is not useful for entrapping drug in the desired amounts. Applicants submit that the Office Action has not provided an explanation as to how this in any way teaches, suggests or motivates one skilled in the art to use ammonium sulphate in a hydration medium for hydrating non-pegylated phospholipids. Further, the phospholipids used in this example are DSPE, which are clearly not the phospholipids used or claimed in the instant invention (the claims recite the use of only three phosphatidyl cholines, which do not include DSPE). Further, presently amended claim 1 does not contain hydrogenated egg phosphatidyl choline as used in Example 7 and 8.

Third, in Kirpotin's example 8 the hydration buffer can be made with many different salts and of all the salts, the patent shows that the use of ammonium sulphate results in low entrapment performance, compared to others -- the best performance being that of polyacrylate. Thus, applicants assert that a person skilled in the art would not choose ammonium sulphate out of all the materials listed in Kirpotin, when its performance is worse than the other materials such as ammonium polyacrylate. Kirpotin indicates a method that does not prefer ammonium ion, and additionally Kirpotin contraindicates the presence of ammonium ion in the hydration media. Where is the motivation to use ammonium ions or ammonium sulphate in the hydration

media when such use of ammonium sulphate has not been shown to be of any advantage, and in fact has shown as a disadvantage over the use of polyacrylate.

Fourth, Example 9 points out that the amount of doxorubicin hydrochloride precipitating in the inner medium is higher when polyacrylate is used as compared to sulphate. This fact also discourages the use of sulphate against polyacrylate and other anions such as citrate, phosphate, DTPA, which were shown to work better for liposome loading.

The Office Action contains no explanation other than a simple statement "that it would be obvious" as to why one skilled in the art after reading Kirpotin would select the choice of hydration medium or inner buffer as ammonium sulphate and sucrose when the reference teaches that sulphate is an inferior anion for liposome loading as compared to the other recited anions. In addition, there is no explanation as to how one skilled in the art from reading Kirpotin would arrive at the use of ammonium sulfate and sucrose in a hydration buffer in the claimed amounts to achieve long-circulating liposomes. Further, the Office Action has provided no reason why one skilled in the art would expect that this use of ammonium sulphate would provide liposomes with a long-circulation time as required by the claims of the instant invention.

Similar data from Kirpotin's Example 1 together with Example 4 also further support the above argument. Kirpotin Example 5 also shows superiority of polyphosphate over sodium sulphate. Kirpotin's teachings clearly discourage the use of ammonium sulphate in a hydration media for hydration of phospholipids, as Kirpotin teach that polymers are better than ammonium/ sodium sulphate/sulphate anion. The Office Action has pointed to nothing that explains why the teaching in Kirpotin would have led the skilled reader to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for hydrating phospholipid would reduce leakage from a loaded liposome and provide a long-circulating liposome that does not contain PEG.

2. Kirpotin does not teach or suggest the use of sucrose in the aqueous hydration media as required by the claims.

Kirpotin does not teach the use of sucrose in the aqueous hydration media. Where is the motivation to use sucrose in the aqueous hydration media as claimed in the present invention in Kirpotin? On Col. 5 and lines 9 to 21 Kirpotin discusses the liposome composition and in particular discusses the charged-polymer precipitating agent:

1. Acidic and basic polysaccharides, both natural and natural-derived, including: polygalacturonates, hyaluronic acid, gum arabic, chondroitin sulfates A, B, and C, keratin sulfates, dermatan sulfates, heparin and its derivatives, pectin and its derivatives, alginic (poly-anhydromannuronic) acid, teichoic acids, chitosans; derivatives of cellulose, amylose, amylopectin, dextran, or other neutral polysaccharide obtained by introduction of carboxyalkyl, phosphate, sulphate, amino-, mono-, di-, trialkylamino, tetraalkylammonium functional groups, derivatives of the said polysaccharides with nitrogen heterocycles, and derivatives obtained by grafting other ionizable functions to polysaccharide backbone.

This list clearly shows that the use of sucrose or neutral saccharide -without any charge- in the hydration medium is not taught or suggested and, in fact, is ruled out. Only derivatives of saccharides are considered as precipitating agents for inclusion in the hydration media. The presence of any other material inside the liposome has not been suggested. Thus, there is simply no teaching or motivation for adding sucrose in the hydration media arising out of Kirpotin.

3. Kirpotin does not teach or suggest removal of the organic solvent after hydration of the liposomes, which is an element of the present claimed invention.

Another element in the claims that is not present in Kirpotin relates to removal of the organic solvent. As pointed out above, Kirpotin's method involves removal of the organic solvent before the hydration. The present claims recite that the solvent can be removed before or after hydrating the lipids and sterols. Nowhere does Kirpotin teach or suggest that the organic solvent could be removed after hydration.

4. Kirpotin does not teach or suggest a method of making long-circulating non-pegylated liposomes.

As already argued in previous responses, applicants believe that the Office Action is over-reaching when it asserts that Kirpotin's mention of using synthetic or naturally occurring phospholipids implies the use of Peg-phospholipids is not necessary. First, synthetic or naturally occurring phospholipids could be pegylated – there is nothing to indicate that they are not pegylated, especially given the fact that every single example in Kirpotin uses pegylated phospholipids. Second, Kirpotin relates to loading liposomes so why would one skilled in the art take a reference in which every method of loading a liposome discussed therein uses pegylated

phospholipids uses to arrive at the claimed invention that does not use pegylated phospholipids (and which further contains other claim limitations not even taught or suggested by Kirpotin). For instance, the present invention relates to a method of making long-circulating liposomes that do not contain pegylated phospholipids, and in addition, the claimed method employs various specific steps and reagents (also not taught or suggested by Kirpotin) to achieve the long-circulating liposomes. The Office Action has not pointed out why one skilled in the art would even look to Kirpotin at all to achieve long-circulating liposomes when Kirpotin does not even address or mention this issue.

The Office Action next states that:

> However since the final product in Kirpotin is a liposome just as in the instant case and since complete hydration of the phospholipid is required for the formation of liposomes, in the absence of showing unexpected results, it is deemed obvious to one of ordinary skill in the art to vary the amounts of the hydrating medium to obtain the best possible results.

Applicants respectfully point out that the liposome product of Kirpotin is not same as in the instant case, because it gives rise to hand foot syndrome on usage. Undeniably, all of the liposomes made in Kirpotin are pegylated liposomes, whereas as per claim 1 of the instant invention the product produced by the method is a non-pegylated liposome. Even assuming the Office Action's argument that the mention of synthetic and naturally occurring phospholipids suggests non-pegylated liposomes, it clearly does not teach or suggest a composition comprising only non-pegylated liposomes.

The difference between Kirpotin and the present invention is not simply "varying the amounts of hydrating medium to obtain the best possible results," as argued in the Office Action. First of all, what are the "best possible results" to which the Office action refers? Kirpotin relates to liposome loading so arguably the "best possible results" relate obtaining a high loading efficiency. In contrast, the present invention relates to obtaining a long-circulating liposome without the use of PEG so arguably the "best possible results" relate to achieving a long lasting stable liposome not using pegylated phospholipids. Where is the argument or evidence to show that it would be obvious to look for what is lacking in Kirpotin relating to liposome loading to combine with another reference relating to entrapment quality of the liposomes (i.e. Papahadjopoulos cited below) to create a long-circulating liposome that does not contain PEG?

The applicants submitted a Declaration showing unexpected results comparing liposomes made by the present invention against liposomes containing pegylated phospholipids (Caelyx). The Office Action has refused to consider these unexpected results and has indicated that the unexpected results need to arrive from the comparison of Kirpotin and liposomes made by the present invention. Since the Office Action states that the liposomes made by the current method should be compared with Kirpotin product, could the Examiner define which is the Kirpotin product to be used for comparison? All examples in Kirpotin are pegylated (so comparison of Caelyx, which is a pegylated liposome, should suffice). Further, which hydration media should be used in the non-pegylated composition that is characteristic of Kirpotin? Which method of loading is to be used - co-precipitation with charged precipitating agent or in the form of pH induced precipitate. Which of the various polymers described in Kirpotin should be used in the hydration media?

There is nothing in the law that states that claimed product has to be compared to the prior art cited by the Office Action. An applicant relying upon a comparative showing to rebut a prima facie case must compare his claimed invention with the closest prior art (and not necessarily the art cited by the Examiner). See *Application of Merchant*, 575 F.2d 865, 869 (CCPA 1978). In this case, the Board rejected an application comparing the claimed invention against a prior art reference and not against the prior art reference cited by the Examiner. The Examiner/Board erroneously required the applicant provide unexpected results over the Examiner's cited art. The CCPA noted that this "approach lacks a basis in law. To apply that approach would place a burden upon the applicant to provide comparison tests of his invention with every cited reference, for each reference may be said to be the "closest" prior art for the particular limitation it allegedly discloses." Applicants respectfully assert that the closest prior art is the liposome product Caelyx (see further discussion of Caelyx below) and as such the Declaration should be considered as it provides the comparison of liposomes made by the claimed method to the closest prior art. Applicants have taken one of the main differences from the prior art (pegylated liposomes) and the claimed invention (non-pegylated) liposomes and compared their circulation times to show that the claimed invention indeed has a long-circulation time and is just as, if not more effective than the prior art pegylated liposomes (Caelyx).

Thus, it is clear that there are many elements in the present claims that are not taught or suggested by Kirpotin.

B. Papahadjopoulos does not teach or suggest each and every element of the claimed invention.

On page 3 of the Office Action it states that:

> Papahadjopoulos discloses methods of formation of liposomes. The methods involve either removal of the organic solvent before hydration (Example 1) or making an emulsion using an organic solvent containing phospholipid and an aqueous medium and evaporating the organic solvent (Example 2). In either method, the amount of lipid is 100 micromoles and the aqueous medium added is 1.5 ml, which corresponds to 15 ml of aqueous medium per millimole of the phospholipid and the hydration medium contains histidine.

Applicant's observations on Papahadjopoulos are as follows. As noted in the summary of the Papahadjopoulos invention, the "invention comprises a method of encapsulating biologically active materials in synthetic, oligolamellar lipid vesicles."

Examples 1 and 2 in Papahadjopoulos are presented in tabular format to help understanding the steps in these examples.

Process steps of Papahadjopoulos	**Applicant's Observations and Comparison to Present invention**
[Phosphatidylglycerol + phosphatidylcholine + cholesterol 10, 40 & 50 μM respectively] dissolved in chloroform, the solvent is evaporated.	Phospholipid contains phosphatidyl glycerol; Initial solvent amount is not given
The thin lipid layer in the flask is then purged with nitrogen and 5 ml of diethyl ether added with stirring to dissolve the lipids. Then 1.5 ml aqueous buffer - pH of 7.4 [10 mM sodium chloride /4 mM of histidine/"TES"(2{[tris(hydroxymethyl)methyl] amino{ethanesulfonic acid] and 10 mg/ml of alkaline phosphatase* is added to form a heterogeneous, 2-phase mixture. The mixture is emulsified by sonication.	The 2nd solvent added is 5 ml and remains in the organic phase when the hydration media is added. The hydration media contains histidine, which is not used in instant invention at the hydration stage. No ammonium sulphate or sucrose is present in the hydration buffer (as required by

	the present invention). The buffer also includes *the biologically active material to be entrapped (i.e. alkaline phosphatase or RNA).
The resulting emulsification is evaporated at 25 °C**/10-50 mm Hg until a viscous gel results. Then 1.5 ml. of the sodium chloride/histidine/TES buffer described above is added to the gel to obtain an aqueous suspension of the gel. The resulting mixture is evaporated at 30 °C/ 10-50 mm Hg / 15 minutes to obtain an opaque suspension of phospholipid vesicles (synthetic liposomes) 0.2 to 0.6μ.	This formation of the gel, diluting with buffer and again evaporating are not present in the present invention. Further, the two-stage addition of aqueous phase is not in the instant invention.

* In Example 2 - 1 mg/ml of ribonucleic acid (RNA) (either polyadenylic acid or 25S tetrahymena ribosomal RNA) instead of alkaline phosphatase.
**In Example 2 – it is 0°C

1. Papahadjopoulos does not teach or suggest "the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present."

Calculation of volume of hydration buffer per mM of lipid in Example 1 and in Example 2

The amount of phospholipid used is 50 μM (cholesterol is 50 μM) and there is 5ml ether in organic phase. In addition, there is 1.5 ml aqueous phase buffer plus 1 ml (– 10mg) from the alkaline phosphatase solution. Although it is not precisely clear how much water of this aqueous phase remains in viscous gel, applicants assume that the maximum is 2.5 ml. In addition, another 1.5 ml of aqueous phase buffer is added to the gel when it changes to liposomes. At that stage the maximum water content would be 4 ml. Thus, 4 ml of aqueous phase for 50 μM converts to 4,000 per 50 mM, or 80 ml per mM, which is clearly outside of the range of the claimed invention. Even if one were to guess that there would be some loss of water during intermediate evaporation, even if assuming that the aqueous phase may be less than 4 ml (such as 3ml), there would be 3 ml of aqueous phase per 50 μM of phospholipid. This converts to 3000 ml per 50 mM; or 60 ml per mM. This is certainly outside the range of the instant invention Even if one calculated that only 2.5 ml aqueous phase remained, it would still be 2500 ml per 50

mM or 50 ml per mM and 2000 ml per 50 mM or 40 ml per mM respectively, which is above the range given in instant invention.

2. Papahadjopoulos does not teach or suggest removal of the solvent before hydration.

The Office Action's statement: "The methods involve either removal of the organic solvent before hydration (Example 1) or making an emulsion using an organic solvent containing phospholipid and an aqueous medium and evaporating the organic solvent (Example 2)" is incorrect. As shown above in the chart, as well as can be seen from reading the Papahadjopoulos patent, in Example 1 there is no removal of solvent before hydration, because the added solvent ethyl ether is present. There is no difference in Example 2 in this respect. Both examples follow the same procedure and the same quantities of the materials are used (the only difference being the type and amount of the biologically active material used). The aqueous level is the same in Example 1 and 2. In other words, Papahadjopoulos does not teach a method of removing all solvent before hydration, to make lipid as of a dry film type before hydration, as in the instant invention. To the contrary, Papahadjopoulos needs the solvent to make an emulsion of water-in-oil when the buffer is added. Therefore, there is no teaching or suggestion in Papahadjopoulos whatsoever that solvent should be removed before adding hydration medium.

3. Papahadjopoulos relates to entrapment quality and has no teaching regarding long-circulating liposomes, let alone even teach or suggest the hydration media in the claimed invention.

Papahadjopoulos correlates ionic strength of buffer with the entrapment quantity of biological material: encapsulation efficiency relates to the ratio of organic phase to aqueous phase and concentration of lipid/phospholipid in the two phase system. However, it neither measures nor correlates any parameter to the increase in circulation time of the liposome prepared and in fact has no such study on circulation time of the liposomes. Just because it was erroneously believed that Papadopoulos has a similar ratio of aqueous phase per phospholipid, it does not mean that it is obvious to one of ordinary skill to think that this ratio will increase circulation time of liposome. It is not possible to predict what process steps give rise to longer-circulation time that results in a greater efficiency in reduction in breast tumor and hence is not

obvious to choose a particular ratio of aqueous phase to phospholipid from one reference for use in another process having altogether different process conditions. Therefore, it is unlikely to motivate one skilled in the art to think of applying a particular range of volume of aqueous hydration medium to the method of the present invention, which is different from that of Papahadjopoulos. In Papahadjopoulos examples 1 and 2, the hydration buffer is sodium chloride/histidine/TES buffer, whereas in the instant invention, the hydration buffer is ammonium sulphate/sucrose. It is not possible to predict what process steps will produce liposome that would give rise to longer circulation time and hence greater efficiency in reduction in breast tumor growth. Therefore, it is not correct to say it is obvious to choose a particular volume of hydration medium of a different composition from that used in another process having altogether different processing conditions to get liposomes having pharmacological properties not reported or referred to, or suggested in that process.

4. Papahadjopoulos does not teach the removal of the solvent before the addition of the aqueous phase.

Since the instant invention also teaches a method wherein the solvent is removed before (or after) the addition of aqueous phase, (both of which give the same results), it is not persuasive to say that the step of removing the solvent before the addition of the aqueous phase is obvious from Papahadjopoulos. It should be noted that in Example 1 of Papahadjopoulos, one solvent is removed but another is added to dissolve the lipids, and the aqueous phase is added to the solution. Papahadjopoulos does not teach the removal of the solvent before the addition of the aqueous phase as argued by the Office Action.

On page 3 of the Office Action it states that:

> Making an emulsion of the phospholipid containing organic solvent and an aqueous medium in the ratios of 1millimole of lipid/15 ml of aqueous medium and removing the organic solvent to form liposomes would have been obvious to one of ordinary skill in the art since Papahadjopoulos teaches that the liposomes can be produced by either process.

Applicants note that the drug to be encapsulated is incorporated in the aqueous phase in Papahadjopoulos procedure, whereas in the instant invention the empty liposomes are first prepared and the drug is loaded afterwards. The procedures are thus quite different and therefore it would not be obvious to one of ordinary skill in the art to think of using the step of removing

the solvent after the liposomes are formed in the process for making non-pegylated long-circulating liposomes without any drug loaded in it.

5. Papahadjopoulos does not teach or suggest use of ammonium sulphate and sucrose as a hydration buffer.

Papahadjopoulos does not teach or suggest use of ammonium sulphate and sucrose as a hydration buffer and further the buffers are internal to the liposomes and are not used to remove extra-liposomal hydration salt, for which instant invention uses a histidine sucrose buffer.

Papahadjopoulos discloses the preparation of liposomes by emulsifying a mixture of lipids in organic solvent and an aqueous mixture of the active material for encapsulation; and then removing the organic solvent and suspending the resultant gel in water. Sucrose is included amongst exemplified active materials for encapsulation (see column 6, lines 31-43) and in the hydration buffer (column 10, lines 53-56) but there is no reference to ammonium sulfate. Thus, there is no teaching of a hydration buffer comprising ammonium sulfate and sucrose.

Papahadjopoulos' method does not say that liposomes can also be made by first removing the organic solvent and then hydrating the phospholipids. It is not obvious to think that it will work that way also.

The argument made in the office action pointing out that in quantitative aspects the instant invention and Papahadjopoulos are the same is not persuasive when they are qualitatively quite different materials: The hydration media is different, the drug is different, and the method of drug loading is different. In a broad sense Papahadjopoulos method of invention calls for the formation of "inverted micelles" in an organic phase and then the removal of organic phase. The system then spontaneously reverts to a bilayer-like structure, with a large amount of aqueous phase encapsulated in large oligolamellar vesicles. Accordingly, the methods are not even similar.

6. Papahadjopoulos does not teach or suggest other numerous claim elements.

Examples 1 and 2 of Papahadjopoulos employ Phosphatidyl glycerol, the use of which is not indicated or claimed in the instant invention. In the instant invention the long circulation effect is achieved without the use of phosphatidyl glycerol; and therefore, the procedure of

Examples 1 or 2 is not relevant to one of ordinary skill in the art to think that a particular range of ratio of aqueous phase to organic phase would lead to long circulating liposome having greater efficiency in reducing breast tumour, without the use of phosphatidyl glycerol.

In Papahadjopoulos, a buffer can be added during the liposome formation (see column 5, lines 30-35 & column 6, lines 5-13) and exemplified buffers include sodium chloride/histidine/2-{[tris(hydroxymethyl)methyl]amino} ethanesulfonic acid (TES) (see Examples 1, 2, 5, & 6). Histidine/TES buffer has been used in Examples 1 and 2 aqueous phase. There is no use of such buffer in the hydration medium in the instant invention.

C. The combination of the teachings of Kirpotin and Papahadjopoulos does not provide the method of claim 1.

1. The combination of Kirpotin and Papahadjopoulos do not teach each and every element of the claims.

Papahadjopoulos or Kirpotin do not teach the claimed ratio of hydration media to phospholipids, nor do they teach removing extra-liposomal salt using a histidine-sucrose buffer, nor do they teach the method using these buffers to make long-circulating non-pegylated liposomes. Not only does the combination of these references fail to teach each and every element, they also fail to suggest the missing elements. The lack of teaching in Kirpotin to derive a process of amended claim 1 is significantly more than the amount of aqueous hydrating medium per mole phospholipid. Even if it is proper to combine the teachings of Kirpotin and Papahadjopoulos (*arguendo*), the combined teaching does not lead the skilled person to conclude that the circulation of non-pegylated liposomes could be prolonged by the use of ammonium sulfate and sucrose in the hydration medium for the production of liposomes to be post-formation loaded with active material. Moreover, Papahadjopoulos does not teach that sucrose would be required if one were to modify Kirpotin Example 8 to use non-pegylated liposomes and load the doxorubicin by the use of ammonium ion gradient. Further, the liposome production process of Papahadjopoulos is so different from that of Kirpotin's that the skilled person would not look to Papahadjopoulos for information as to the amount of hydration media to use. Kirpotin is concerned with loading pre-formed liposomes with the active material for encapsulation whereas Papahadjopoulos is concerned with encapsulation of the active material during liposome formation and hence, when considering patentability of the amended claims, there is no reason

for combining the teachings Kirpotin and Papahadjopoulos. Moreover, there is no reasonable expectation of success in producing the invention of the amended claims by combining those teachings.

As demonstrated by data in the application, the loaded non-pegylated liposomes of the invention are at least as effective (see US 2005/0142178 at paragraphs 0096, 0097, 0166, & 0199), have lower toxicity (see US 2005/0142178 at paragraphs 0095, 0117, & 0253) and comparable plasma half life (see paragraph 0255) compared with pegylated liposomes. The Office Action has dismissed this data because it is based on a commercially available pegylated product (CAELYX; also known as DOXIL or DOX-SL) and not on Kirpotin. However, it is stated in Emanuel paragraph 0033 (discussed *infra*) that CAELYX does contain ammonium sulfate and sucrose. Accordingly and having regard to the objective of the present invention (*viz.* to provide a non-pegylated liposome that is as effective as CAELYX but with reduced toxicity), the comparison with CAELYX is appropriate and meaningful. It establishes the advantage of the non-pegylated liposomes of the present invention over pegylated liposomes containing both ammonium sulfate and sucrose that could not have been predicted from Kirpotin and Papahadjopoulos, particularly that observed in efficiency in reduction in breast tumor, which is unexpected. The applicants feign no hypothesis for this performance of the instant invention and believe that it is the result of the concerted process.

Until recently, the potential of liposomes as drug carriers has been limited by the rapid clearance of liposomes from the bloodstream. For example, conventional liposomes may be largely cleared from the bloodstream within 1-2 hours after intravenous administration (See Saba, US patent 5,395,619). Thus, a variety of approaches for extending the circulation time of liposomes have been proposed. Two of these have been successful in extending the half life of liposomes in the bloodstream by periods of up to 40-50 hours. In one approach, described in co-owned U.S. Pat. No. 4,837,028, liposomes are formulated with the ganglioside G_{M1} and predominantly rigid lipids. In another general approach, disclosed in co-owned U.S. Pat. No. 5,013,556, liposomes are coated with a layer of polyethylene glycol (PEG) chains.

The Hong Paper cited by Office Action later in the office action (and discussed again below) shows that there is nothing like a standard pegylated liposome. The pharmaceutical properties of pegylated or non-pegylated liposomes vary from preparation to preparation. Amongst the pegylated liposomes the extent of pegylation, the molecular weight of PEG used,

and the manner of pegylation make a difference. Thus, there is no point in insisting that the comparison of the product of the instant invention should be made with Kirpotin product. The internal comparison with a comparative example wherein most of the steps and materials are same is the best comparison (as with Caelyx). When the Office Action has cited many prior art documents as the so called closest prior art documents it would require applicant to compare his product with products each of these patents. In fact, the Office Action has required such comparison in its refusal to consider the applicant's Declaration for unexpected results over Kirpotin and Forssen and Janoff (see rejection made in paragraph 6 of the Office Action requiring unexpected results over Forssen and Janoff).

III. Claim Rejections – 35 U.S.C. § 103(a) – Kirpotin/Papahadjopoulos/Emanuel.

In the Office Action in paragraph 5, page 4, claim 62 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Kirpotin and Papahadjopoulos in further combination with Emanuel (US 2002/0151508). Having regard to the amendment now made to the claims and the comments below, Applicants respectively submit that the present claims are patentable under 35 U.S.C. § 103(a) and in particular are not obvious over any combination of the teachings of Kirpotin, Papahadjopoulos and Emanuel.

Kirpotin and Papahadjopoulos have been discussed above and applicants have shown that the present invention is not obvious from their combination as that they do not teach nor suggest each and every element of the claim. The Office Action has argued that Emanuel shows routine use of ammonium sulfate-histidine-sucrose buffer in the preparation of liposomes.

In Papahadjopoulos, a buffer can be added during the liposome formation (see column 5, lines 30-35 & column 6, lines 5-13) and exemplified buffers include sodium chloride/histidine/2-{[tris(hydroxymethyl)methyl]amino} ethanesulfonic acid (TES) (see Examples 1, 2, 5, & 6). Histidine/TES buffer has been used in Examples 1and 2 aqueous phase. However as discussed above, there is no use of an ammonium sulfate-sucrose hydration buffer as required in the instant invention nor is there use of a histidine sucrose buffer to remove extra-liposomal salt.

The present Office Action points out that Papahadjopoulos uses histidine buffer extensively. It is not obvious from extensive use of histidine buffer in Papahadjopoulos, that a histidine sucrose buffer should be used to remove extra-liposomal salt from the liposomes after sizing, which is a limitation in the claimed invention. The method of the instant invention

consists of steps in a certain order, and which step use a histidine sucrose buffer is a defined element of the present invention.

Emanuel does not teach or suggest a hydration buffer comprising ammonium sulfate and sucrose nor does it teach or suggest a buffer comprising histidine and sucrose for removal of extraliposomal salt after sizing of the liposomes.

According to the Office Action, the use of sucrose in a buffer becomes obvious on reading Emanuel after considering Kirpotin and Papahadjopoulos. Applicants have clearly explained above that there are two types of aqueous phases used in the instant invention: one is a hydration media that comprises ammonium sulphate and sucrose, and the other is extra liposomal hydration salt removing buffer and/or drug loading buffer. These two buffers are different and serve different purposes and as such are not interchangeable. When to use which buffer is not obvious from Emanuel. Further, Emanuel does not suggest that any buffer is to be made or used. It only says that the histidine, sucrose and ammonium sulphate are present in the product. It also says that the product contains pegylated phospholipids (not present in the claimed invention). Thus, Emanuel does not teach or suggest <u>any</u> of the buffers or the steps of present claimed invention and as such does not cure the deficiencies in Kirpotin and Papahadjopoulos. As discussed above, Kirpotin does not direct to use ammonium sulphate in the hydration medium; and does not show the use of ammonium sulphate in the hydration medium in the preparation of non-pegylated liposomes. Papahadjopoulos uses more than double the amount of hydration media that the instant invention uses; in a different method where hydration is performed twice. Finally Emanuel does not describe the process at all. Under this situation it is hard for one skilled in the art to imagine the instant invention. This thinking is best expressed as hind sight logic to build a story for obviousness after reading the instant invention.

Emanuel relates to the use of liposomal anthracycline compositions in association with a growth factor receptor inhibitor to treat breast cancer and other proliferative diseases. The preferred liposomal anthracycline composition is pegylated liposomal doxorubicin comprising *inter alia* histidine, ammonium sulfate and sucrose (see paragraphs 0010 –0015; 0033 & Claims 4 & 5).

Emanuel reports that ammonium sulfate, histidine and sucrose are all present in DOXIL but no details are provided as to how DOXIL is manufactured. However, it appears that at least

the bulk of the components are contained in the liposomes and there is no reason to assume that the histidine and sucrose were ever used to remove extra-liposomal ammonium-ion.

Emanuel paragraph 033 describes Doxil® as a liposomal dispersion in a vial. The composition of STEALTH® has MPEG-DSPE – a Pegylated phospholipid, HSPC and cholesterol encapsulating 90 % of the drug doxorubicin hydrochloride. Each ml of the dispersion also contains ammonium sulphate, histidine as a buffer, HCl and or NaOH for pH control; and sucrose to maintain isotonicity. 10% of the drug is outside the liposomes.

This shows the use of the histidine buffer for preserving pH of the liposome in the vial. The sucrose is added for isotonicity, as noted in the paper, and it is not same as use of histidine-sucrose buffer as in Claim 1, for removing extra-liposomal salts. This is not obvious by reading in the above paragraph.

In the instant invention histidine sucrose buffer is employed to remove extra-liposomal salt of the hydration medium that is ammonium sulphate whereas in Emanuel, it is included in the buffer that has to be inside the liposome.

In addition, Applicants also note as shown above that neither Kirpotin, Papahadjopoulos nor Emanuel disclose the use of histidine and/or sucrose to remove extra-liposomal hydration salt from liposomes.

In addition, as mentioned previously, it is improper, when considering patentability of the amended claims, to combine Kirpotin and Papahadjopoulos because Kirpotin is concerned with loading preformed liposomes with the active material for encapsulation whereas Papahadjopoulos is concerned with encapsulation of the active material during liposome formation.

In the relevant example of Kirpotin (Example 8), the outer buffer contains neither histidine nor sucrose, in contrast to the claimed invention where both histidine and sucrose are used to remove extra-liposomal salt.

In the process of Papahadjopoulos, buffers, including a sodium chloride/histidine/TES buffer, can be used but there is no reference to the use of sucrose as a buffer or to any reference to the use of any ammonium salt. Further the buffers are internal to the liposomes, and are not used to remove extra-liposomal hydration salt.

Accordingly, the process steps of the instant invention are not disclosed and not at all obvious from Emanuel or the combination of the three cited references.

IV. Claim Rejections – 35 U.S.C. § 103(a) – Forssen/Papahadjopoulos/Janoff

In paragraph 6 on page 5, claims 1-8, 10, 12, 14-22 and 62 are rejected under 35 U.S.C. 103(a) as being unpatentable over Forssen (US patent 5,714,163) in combination with Papahadjopoulos and Janoff (US patent 4,880,635). Having regard to the amendment now made to the claims and the comments below, Applicants respectively submit that all claims now in the Application are patentable under 35 U.S.C. § 103(a) and in particular are not obvious over any combination of the teachings of Forssen, Papahadjopoulos and Janoff.

Papahadjopoulos has been discussed above and applicants have shown that Papahadjopoulos does not employ the volume of the hydration buffer/medium as in instant invention and further, that it does not remove solvent before addition of hydration buffer/medium. Papahadjopoulos does not use ammonium sulphate and sucrose in the hydration medium. In addition, no step in the instant invention is made obvious by Papahadjopoulos or by the combination of the cited references.

The Office Action has stated that:

> Forssen discloses a method of preparation of liposomes wherein the lipid film is hydrated with ammonium sulphate. The liposomes contain DSPC , cholesterol and vincristin. Vincristin is added to pre-formed liposomes (Example 1). Although Forssen teaches the use of 300 mM of sucrose, he does not teach the use of hydration medium containing both ammonium sulphate and sucrose. What is lacking in Forssen is the method of preparation of liposomes first forming an emulsion of the organic solvent and an aqueous medium and the removal of the organic solvent and also lacking is the claimed amount of the hydrating medium per mM of phospholipid.

A. Forssen does not teach or suggest a hydration buffer containing ammonium sulfate and sucrose.

In Forssen, Example 1 spray-dried distearoylphosphatidylcholine/cholesterol (DSPC/CHOL) lipid was hydrated with either a buffer containing the ammonium salt of one of the counterions or 300 mM sucrose. (Emphasis added). One of the 10 buffers used contained ammonium sulfate. After sonication, annealing, centrifuging and buffer exchange by gel filtration on a Sephadex column previously equilibrated with unbuffered 300 mM sucrose, vincristine was ion-exchange loaded by incubation of the liposomes with an aqueous solution of its sulfate salt.

Forssen relates to phosphatidyl choline/cholesterol (PC/CHOL) liposomes containing vincristine or other cationic vinca alkaloids and an anion in an aqueous phase of liposome. Reference is made to several prior art methods for forming liposomes and there is no suggestion that Forssen teaches any new or modified method of liposome formation. It is stated that a significant benefit of the liposomes used by Forssen is that they can be prepared without a transmembrane or pH gradient (see column 4, lines 26-30). In the generally exemplified process, PC/CHOL is hydrated with aqueous anion and the vinca alkaloid is loaded by ion-exchange loading (see column 4, line 49 to column 5, line 10).

The following line of Example 1 describes what can be used in the buffer in the Forssen process: "Vesicles were prepared by hydrating approximately 500 mg of sprayed-dried lipids, DSPC:Cholesterol (2:1, mole ratio), at 65°C with either a buffer containing the ammonium salt of one of the counterions or 300 mM sucrose." Emphasis added

Applicants direct the Examiner to Table 1 of Example 1 of Forssen (col. 6, line 53 to col.7 line - 14). When the counter ion is tartrate, the percent of vincristin entrapped is 90 % and when the counter ion is sulphate, the percent of vincristin entrapped drops to 49 %. This table shows that different counterions have different entrapment rates. The Office Action has not explained why one skilled in the art looks at Example 1 of Forssen would choose sulphate as a counter ion especially when there are other ions (except succinate) giving entrapment above 67 %? Further, Table 1 has no data for vcr entrapment when "sucrose" is used (not along with counter ion). Thus there is no teaching here of a buffer comprising ammonium sulphate and sucrose.

If one reads the same example completely, further down in the animal experiment there is a sentence in col. 7 lines 26 – 32 as follows:

> The mice were randomized into 11 treatment groups and therapy was initiated four days after tumor implantation. The chemotherapeutic treatment groups consisted of free Vcr and nine vesicle-Vcr formulations (Vcr salts of glutamate, tartrate, hydrogen diphosphate, aspartate, EDTA, succinate, pyrophosphate, lactobionate, and citrate). Dosing was at 2.5 mg/kg. Tumor-bearing untreated controls received a treatment of 300 mM sucrose in a volume equivalent to the experimental groups.

Notably from this passage, sulphate is not at all used, which clearly shows that the inventors did not think sulphate was useful.

There is no teaching in Forssen that would have led the skilled reader to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for hydrating phospholipid would reduce leakage from a loaded liposome. Moreover, there is no reason to conclude from Forssen that sucrose would be required for decreasing leakage or prolonging circulation and/or increasing efficiency in reduction in breast tumor if doxorubicin is loaded by the process of the present invention.

On page 6 of the office action, the Office Action writes:

> To include sucrose in the hydration medium of Forssen would have been obvious to one of ordinary skill in the art since such a procedure would enable the presence of sucrose within the liposomes as well as outside and since Janoff teaching that the liposomes retain the active agent during dehydration and rehydration procedures.

The fact that sucrose or other sugar inhibits leakage of an encapsulated active material from dehydrated liposomes is no indication that it will prevent leakage from the liposomes prior to dehydration or from the rehydrated liposomes after loading with the required drug or other active material.

Even if sucrose-histidine buffer is used for washing extraliposomal hydration salts, it introduces sucrose in the outside layer of liposome, it is an ingenious technique of getting sucrose in the outside layer. And it is not an obvious process step, and by itself has inventive merit, in achieving removal of hydration media salts and depositing sucrose on outside layer of the liposome after the liposomes are sized.

B. The combination of Papahadjopoulos does not teach each and every element of the claimed invention.

The Office Action repeats the argument that:

> Papahadjopoulos discloses methods of formation of liposomes. The methods involve either removal of the organic solvent before hydration (Example 1)or making an emulsion using an organic solvent (Example 2). In either method, the amount of the lipid is 100 micromoles and the aqueous medium added is 1.5 ml which corresponds to 15 ml of aqueous medium per millimole of phospholipid.

As shown above, in both Examples 1 and 2 of Papahadjopoulos, the solvent chloroform is removed but ethyl ether is added to dissolve the lipids, and the ethyl ether is not removed.

Therefore this statement in the Office Action's Office Action is erroneous. In both the examples phosphatidyl glycerol (which is outside the scope of instant invention) is used, and further the procedure of obtaining liposomes is through a gel formation stage, which is not at all present in the claimed invention.

Above, applicants have shown that the Office Action's calculation of 15 ml/mM of hydration medium used in Papahadjopoulos is not correct. This reference therefore does not fill up the gap as shown by "what is lacking in Forssen."

C. Janoff does not teach or suggest the hydration media claimed in the present invention.

On page 6 of the office action, the Office Action writes:

> Janoff teaches that sugars such as sucrose when present both inside and outside would enable the liposomes to retain Adriamycin during dehydration and rehydration (col. 21, line 23 through col. 21 line 27). Janoff further teaches the hydration of 80 micromoles of lipid with 21 ml of buffer (25 ml per mmole).

Janoff discloses the preparation of dehydrated liposomes by drying liposome preparations under reduced pressure in the presence of one or more protective sugars. Exemplified sugars include sucrose. The dehydration is conducted under vacuum with or without prior freezing of the liposome preparation. There is no reference to any ammonium salt or to any sulfate. Reference is made to loading rehydrated liposomes using a concentration gradient created after rehydration. In the exemplified processes, 80 μmoles EPC was hydrated with 2 ml aqueous solution containing 150 mM sodium chloride, 20 mM HEPES and the respective sugar (see column 8, lines 40-63).

The fact that sucrose or other sugar inhibits leakage of an encapsulated active material from dehydrated liposomes is no indication that it will prevent leakage from the liposomes prior to dehydration or from the rehydrated liposomes after loading with the required drug or other active material. Further, there is no evidence to say that leakage of liposomes has any relation with long-circulation of liposomes.

It is interesting to note that the Janoff patent was first published as a PCT 86/1103 on February 27, 1986. Subsequently Mayer filed a patent on March 5, 1987 (see WO8806442(A1) 1988-09-07) and referred to Janoff noting that sucrose can be used for dehydration. Mayer did

not think of using sucrose in a hydration medium for hydrating phospholipids in making liposomes. Thus such use was not obvious from Janoff. Even as on the date of filing the instant application, December 31, 2002 - more than 15 years after Janoff publication - nobody has used sucrose in the medium for hydration of phospholipids. This fact clearly establishes that it is not obvious to think that sucrose in combination with ammonium sulphate can be used in the phospholipid hydration medium for getting leak-proof liposomes and long-circulating non-pegylated liposomes.

The combination of the teachings of Forssen, Papahadjopoulos and Janoff does not provide the process of amended Claim 1. The lack in the teaching of Forssen to derive a process of amended claim 1 is significantly more than the combined use of ammonium sulfate and sucrose and the amount of aqueous hydrating medium per mole phospholipid. Even if it is proper to combine the teachings of Papahadjopoulos with that of Forssen and Janoff (*arguendo*), the combined teaching of these three references does not lead the skilled person to conclude that the circulation of non-pegylated liposomes could be prolonged by the use of ammonium sulfate and sucrose in the hydration medium for the production of liposomes to be post-formation loaded with active material using a transmembrane gradient. In particular, neither Papahadjopoulos nor Janoff provides the skilled reader of Forssen with the information that the use of both ammonium sulfate and sucrose in an aqueous hydration medium for preparing non-pegylated liposomes would reduce leakage from a loaded liposome and prolong circulation thereof. Papahadjopoulos does not teach that sucrose would be required if Forssen Example 1 was to be modified to load the vincristine by use of ammonium ion gradient and Janoff is only relevant if it is required to store the liposomes before loading. If it was desired to prolong the circulation time of the Forssen vinca alkaloid-loaded liposomes, the teaching of the art at the date of filing the present application was to pegylate the liposome not to seek to modify the lipid hydration media.

Papahadjopoulos is specifically concerned with incorporating the active material during liposome formation whereas Forssen and Janoff are specifically concerned with the loading of active material into formed liposomes and hence, when considering patentability of the amended claims, there is no reason for combining the teachings of Papahadjopoulos with either Forssen or Janoff. Moreover, there is no reasonable expectation of success in producing the invention of the amended claims by combining those teachings.

Forssen does not recommend use of ammonium sulphate in hydration media, he does not even show use of sucrose with ammonium sulphate; Papahadjopoulos does not recommend the volume of hydration media as in instant invention, nor does he show solvent removal before hydration; and Janoff does not suggest the addition of sucrose to the hydration media with ammonium sulphate. None of these three suggest any effect of such liposomes on long-circulation, and they are thus not a relevant combination, and do not make the present invention obvious when taken together.

D. Maruyama and Park

The Office Action has cited Maruyama *et al* Int. J. Pharmac 111 (1994) 103-107 (Maruyama) and Park *et al* Biochem Biophys Acta 1108 (1992) 257-260 (Park) in support of a contention that other means were known for prolonging circulation time. As explained below the relevant disclosures of both Maruyama and Park are limited to specific negatively-charged phospholipids and do not teach any expectation for other negatively-charged phospholipids. What Maruyama and Park actually teach is that, in general, no prediction can be made of the effect on circulation time of using negatively-charged phospholipids.

Maruyama reports the effect on prolonging liposome circulation of various phosphatidyl polyglycerols (DPP-PG). The investigation of DPP-PGs was predicated on the knowledge that pegylated liposomes obtained by incorporation of polyethylene glycol derived phosphatidylethanolamine (PEG-PE) have increased liposome blood levels and reduced RES cell liposome uptake, thereby increasing circulation time.

Maruyama and Park teach that blood circulation time is prolonged on using negatively charged phospholipids. The amended claim 1 excludes all negatively charged phospholipids, and thus these references are not relevant.

In Maruyama, the liposomes were prepared from DSPC, Cholesterol and the respective DPP-PG. The increased circulation time was attributed primarily to the presence of negatively charged DPP-PG in the lipid bilayers and low aggregation resultant from small liposome size. However, it is reported that the presence of negatively charged phospholipids in other liposomes have reduced circulation time (see paragraph bridging pages 105 & 106). Accordingly, Maruyama does not teach that, as a generality, the presence of negatively charged phospholipids will prolong circulation time. The only conclusion made is that DPP PG-coated liposomes had a

longer circulation time than liposomes lacking DPP-PG. Chemically, polyglycols differ from polyethylene glycol in respect of the number and frequency of hydroxyl groups in the polymer chain and accordingly those skilled in the art will understand the term "non-pegylated liposome" as excluding liposomes incorporating polyglycol-derivatized lipids.

Park reports the effect on liposome circulation time by incorporation into EPC/CHOL liposomes of negatively charged phospholipids obtained by coupling aliphatic dicarboxylic acids to dioleoyl phosphatidyl ethanolamine (DOPE). It is reported that the effectiveness of the negatively charged derivatives in prolonging liposome circulation was found to be dependent upon the length of the hydrocarbon chain between the amide and terminal carboxyl group. However, prior art reports that liposomes containing negatively charged phospholipids have decreased circulation time and are localized mainly in the liver and spleen (see paragraph bridging the columns on page 257 & last complete paragraph on page 258) are acknowledged. The only conclusions made in Park are: (i) that the effectiveness of aliphatic dicarboxylic coupled DOPE in prolonging circulation is dependent upon the length of the hydrocarbon chain between the amide and terminal carboxyl group; and (ii) that not all negatively charged phospholipids enhance liposome uptake by RES, some even reduce the uptake (see abstract).

Maruyama and Park teach that blood circulation time is prolonged on using negatively charged phospholipids. Claim 1 excludes all negatively charged phospholipids, and so the citations of these references are irrelevant.

V. Claim Rejections – 35 U.S.C. § 103(a) – Forssen/Papahadjopoulos/Janoff/Emanuel

In paragraph 7, page 8 claim 62 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Forssen (5,714,163) in combination with Papahadjopoulos (US patent 4,235,871) and Janoff (US patent 4,880,635) further in view of Emanuel(US 2002/0151508). Having regard to the amendment now made to the claims and the comments below, Applicants respectively submit that Claim 1, which has been amended to include the limitations of claim 62 (and all other claims) now in the Application are patentable under 35 U.S.C. § 103(a) and in particular are not obvious over any combination of the teachings of Forssen, Papahadjopoulos, Janoff and Emanuel.

The combinations of the cited references do not teach or suggest each and every element of the claims.

Forssen, Papahadjopoulos, Janoff, and Emanuel have been discussed above. Claim 1, which requires sizing the liposomes to about 0.06 μm and the use of a sucrose-histidine buffer solution to remove extra-liposomal hydration salt, is patentable. Moreover, none of Forssen, Papahadjopoulos, Janoff or Emanuel discloses the use of histidine and/or sucrose to remove extra-liposomal hydration salt from liposomes. Neither Forssen nor Janoff refer to the use of a buffer containing histidine or sucrose but, as mentioned above, Papahadjopoulos & Emanuel do disclose a histidine buffer. However, the Papahadjopoulos buffers do not contain sucrose and both the Papahadjopoulos and Emanuel buffers are internal to the liposomes and not used to remove extra-liposomal hydration salt.

In addition, as mentioned previously, it is improper, when considering patentability of the amended claims, to combine Papahadjopoulos with Forssen or Janoff because Papahadjopoulos is specifically concerned with incorporating the active material during liposome formation whereas Forssen and Janoff are specifically concerned with the loading of active material into formed liposomes.

VI. Claim Rejections – 35 U.S.C. § 103(a) – Forssen / Papahadjopoulos / Janoff / Radhakrishnan or Uchiyama.

In paragraph 8, page 8, claims 1-8, 10-22 and 61-62 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Forssen (5,714,163) in combination with Papahadjopoulos (4,235,871) and Janoff (4,880,635), further in view of Radhakrishnan (5,192,528) or Uchiyama (International Journal of Pharmaceutics, 1995). Having regard to the amendment now made to the claims and the comments below, Applicants respectively submit that all claims now in the Application are patentable under 35 U.S.C. § 103(a) and in particular are not obvious over any combination of the teachings of Forssen, Papahadjopoulos, Janoff, Radhakrishnan and Uchiyama.

A. Radhakrishnan does not teach each and every element of the claimed invention, or cures the deficiencies in the other cited references.

Forssen, Papahadjopoulos and Janoff have been discussed above. Radhakrishnan discloses the use of aerosolized aqueous suspensions of corticosteroid for inhalation to treat respiratory tract conditions or diseases. The exemplified processes for liposome preparation are by solvent-injection (see column 5, lines 5-15 & Example 1A) and lipid film hydration (see column 5, lines 16-29 & Examples 1B & 2). In each case, the corticosteroid is entrapped during liposome formation. There is no disclosure of the presence of ammonium sulfate or sucrose in an aqueous hydration media or of the loading of corticosteroid into preformed liposome.

B. Uchiyama does not teach each and every element of the claimed invention, or cures the deficiencies in the other cited references.

Uchiyama reports the effect of size and fluidity of liposomes on their accumulation in tumors. The liposomes were prepared from EPC or hydrogenated EPC (HEPC), dicetyl phosphate (DCP) and CHOL in the molar ratio 5:1:4 by hydration with an isotonic phosphate buffer (not ammonium sulfate and sucrose as required by the present invention). See page 196-197. It was concluded that accumulation of liposome from the vascular space into a tumor is primarily governed by their size and not by their membrane fluidity or blood circulation time.

The Office Action's calculation of the amount of aqueous hydration media per mmole phospholipid from Uchiyama (incorrectly) assumes that all lipids are phospholipids. However, 40 mole percent of the lipids is provided by CHOL. Accordingly, the 5 ml phosphate buffer was added to 200 μmole lipid of which 60% was provided by the phospholipids (EPC/HEPC & DCP) and hence the relative amount of hydration media is 41.5 ml per mmole phospholipid. This is outside the range required by the claims.

For reasons explained above, there is no reason, when considering patentability of the amended claims, for combining Papahadjopoulos with either Forssen or Janoff and, even if there was (*arguendo*), no reason from the combination of the teachings of Forssen, Papahadjopoulos and Janoff to expect that the use of both ammonium sulfate and sucrose in the process as defined in amended Claim 1 would decrease leakage from loaded non-pegylated liposomes and provide a product that was comparable with loaded pegylated liposomes and which would have a reduced toxicity. This lack of teaching is not resolved by either Radhakrishnan or Uchiyama. Neither Radhakrishnan nor Uchiyama purports to contribute anything new to the production of liposomes or mentions either ammonium sulfate or sucrose. The patentability of the amended claims does

not depend solely on the amount of aqueous hydrating medium used and accordingly the mere fact that Radhakrishnan (but not Uchiyama) does disclose amounts within and overlapping with that required by claim 1 does not provide the missing information to lead to a process in accordance with amended Claim 1.

VII. Claim Rejections – 35 U.S.C. § 103(a) – Hong/Papahadjopoulos/Janoff

In paragraph 9, page 9, claims 1-8, 10, 12, 14-22 and 62 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Hong (Clinical Cancer Research, 1999) in view of Papahadjopoulos (4,235,871) and Janoff (4,880,635) cited above. Having regard to the amendment now made to the claims and the comments below, Applicants respectively submit that all claims now in the Application are patentable under 35 U.S.C. § 103(a) and in particular are not obvious over any combination of the teachings of Hong, Papahadjopoulos and Janoff.

Hong does not teach each and every element of the claimed invention, or cures the deficiencies in the other cited references.

Papahadjopoulos has been discussed above. Janoff has been discussed above and with further comment provided below. The Hong Paper cited by Office Action shows that there is nothing like a standard pegylated liposome. The pharmaceutical properties of pegylated or non-pegylated liposomes vary from preparation to preparation. Amongst the pegylated liposomes the extent of pegylation, the molecular weight of PEG used, the manner of pegylation make difference, and there is no point in insisting that the comparison of the product of the instant invention should be made with Kirpotin product. The internal comparison with comparative example wherein most of the steps and materials are same is the best comparison. When Examiner has cited many prior art documents so called close prior art documents – primary reference used in combinations like Kirpotin, Forssen and Hong, it would require applicant to compare his product with products each of these patents.

Hong can not be combined with Papahadjopoulos because the very purpose of combination of Papahadjopoulos was to show that it uses the same volume of hydration media as in the instant invention, has been shown to be incorrect (see discussion above). Thus, the combination with Janoff has been shown as futile. Therefore it is not relevant to say that the instant invention is obvious from such a combination. Further, the additions of Radhakrishnan

and/or Uchiyama are of no use as the secondary and tertiary references failed to show any desired steps that were essential for showing obviousness of the instant invention.

In addition, there is no teaching in Hong that would have led the skilled reader to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for hydrating phospholipid would reduce leakage from a loaded liposome. Further, there is no evidence to say that leakage of liposomes has any relation with long circulation of liposomes, as required by the claims.

Hong's experiment as described employs DSPC: Cholesterol at a 3:2 molar ratio; with variable PEG-DSPE. The contents were hydrated at 55 °C in ammonium sulphate solution (250 mM at pH 5.0) and extruded through polycarbonate membrane filters of 0.1 to 0.05 μm pore size. The elements in claim 1 of the instant invention that are not taught or suggested by Hong are reproduced and are underlined below:

> 1) removing the solvent or mixture of solvents before or 2) after hydrating the lipids and sterols; 3) sizing the non-pegylated liposomes formed to about 0.06 μm to form a liposomal composition; 4) removing extraliposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution; 5) wherein the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present to form non-pegylated liposomes and wherein the aqueous hydration media comprises ammonium sulfate and sucrose and 6) wherein the non-pegylated liposomes have a blood circulation half life of at least 25 times longer than conventional non-liposomal formulations when tested in Swiss albino mice at equivalent doses.

The combination of the teachings of Hong, Papahadjopoulos and Janoff does not provide a process of amended claim 1. The lack in the teaching of Hong to derive a process of amended claim 1 is significantly more than the combined use of ammonium sulfate and sucrose and the amount of aqueous hydrating medium per mole phospholipid. Even if it is proper to combine the teachings of Papahadjopoulos with that of Hong and Janoff (*arguendo*), the combined teaching of these three references does not lead the skilled person to conclude that the circulation of non-pegylated liposomes could be prolonged or the efficiency of reduction in breast tumors by the use of ammonium sulfate and sucrose in the hydration medium for the production of liposomes to be post-formation loaded with active material. In particular, Hong specifically teaches that

liposome circulation can be prolonged by using pegylated liposomes and neither Papahadjopoulos nor Janoff teaches or suggest that the use of both ammonium sulfate and sucrose in an aqueous hydration medium for preparing non-pegylated liposomes would reduce leakage from a loaded liposome and prolong circulation thereof. As mentioned above, the fact that, as reported by Janoff, sucrose or other sugar inhibits leakage of an encapsulated active material from dehydrated liposomes is no indication that it will prevent leakage from the liposomes prior to dehydration or from the rehydrated liposomes after loading with the required drug or other active material. Unless it was required to dehydrate the Hong liposomes for storage prior to loading, there would be no reason taught by Janoff to include a sugar in the liposome.

Hong and Janoff are concerned with loading preformed liposomes with the active material for encapsulation whereas Papahadjopoulos is concerned with encapsulation of the active material during liposome formation and hence, when considering patentability of the amended claims, there is no reason for combining Papahadjopoulos with Hong and/or Janoff.

In Janoff, extrusion technique vesicles (ETVs) were prepared using a solute solution containing adriamycin and 250 mM trehalose. The samples were dehydrated for 24 hours without prior freezing. The adriamycin content of the initial sample and the rehydrated vesicles was determined as described in Example 1.

As clearly demonstrated by this Example, the sugar trehalose is capable of protecting liposomes during dehydration and subsequent rehydration so that more than 90% of the material encapsulated within the liposomes is still retained therein after rehydration.

The Office Action states that Janoff teaches addition of sucrose to the phospholipid hydration HEPES buffer while preparing vesicles for protection of liposome membranes during dehydration and rehydration. The effect of such sugar addition while preparing vesicles is performed with certain buffers. In Example 1, ETVs are used and the concluding lines (Col. 11, lines 31 – 38) are as follows:

> The results of these experiments are shown in Table 1. As shown therein, more than 90% of the drug is retained following dehydration and rehydration, i.e. the same levels as those achieved with $^{22}Na^{+}$ and ^{3}H-inulin. Moreover, the rate of leakage of adriamycin from the rehydrated vesicles is comparable to the rate observed with vesicles which have not been dehydrated (see Bally, et al., (1985), Biochim. Biophys. Acta., 812:66).

Applicants respectfully point out there is no need for dehydration or rehydration of liposomes, which is the case in the instant invention, and accordingly there is no need to add sugar according to Janoff. Janoff does not suggest that sugar addition will increase the circulation time. See Col.11, lines 39 – 43 noting that: "As clearly demonstrated by this Example, the sugar trehalose is capable of protecting liposomes during dehydration and subsequent rehydration so that more than 90% of the material encapsulated within the liposomes is still retained therein after rehydration." This conclusion also says that trehalose offers protection during dehydration and rehydration to materials encapsulated within the liposome and has nothing to do with increasing circulation time. It does not say that when liposomes do not go through dehydration or rehydration, trehalose will offer protection or not.

Further the results of experiments in Example 7 of Janoff leads to the conclusion:

> As shown by these results, well over 80% of the internal contents of each of the three types of liposomes were retained after the dehydration/rehydration process without the use of any protective sugars. Moreover, adding trehalose to these types of liposomes somewhat decreased, rather than increased, the amount of internal contents retained in the liposomes after the dehydration/rehydration process. Col.16, lines 37 – 44.

This paragraph actually shows that the trehalose decreased the amount of entrapment. Thus it is not proper to say that by reading Janoff a person having ordinary skill in the art will think that addition of sugar to ammonium sulphate containing hydration medium when there is no hydration and dehydration of liposomes, would produce liposomes having a long-circulation time.

It should be remembered that all inventions are made from known things and facts. Just knowing that sugar is a material that makes liposome membranes less permeable, does not make it obvious to make liposomes with long circulation time and increase efficiency of reducing breast tumor when it is loaded with doxorubicin. All other factors including the materials used, each and every step in the preparation of such liposome contributes to make the invention. It is the concerted effect of the total process that gives the unexpected results.

Janoff does not show the addition of sucrose/sugar to the hydration media for getting it inside the liposome, and there is no motivation for doing so by reading Janoff --its abstract clearly says it is for stabilizing liposomes during hydration and rehydration.

The Office Action also states that Janoff teaches the use of a hydration media at 25 ml per mM of phospholipid. What is the hydration media? Sodium chloride, HEPES and trehalose buffer; what is the phospholipid? Egg phosphatidyl choline. This reference to 25ml per mM is not relevant to the conditions in instant invention – the phospholipids are different and the hydration media is different.

> On col. 5 line 54-68, Janoff writes: ". . . So that the liposomes will survive the dehydration process without losing a substantial portion of their internal contents, it is important that one or more protective sugars be available to interact with the liposome membranes and keep them intact as the water in the system is removed. A variety of sugars can be used, including such sugars as trehalose, maltose, sucrose, glucose, lactose, and dextran. In general, disaccharide sugars have been found to work better than monosaccharide sugars, with the disaccharide sugars trehalose and sucrose being most effective. Other more complicated sugars can also be used. For example, aminoglycosides, including streptomycin and dihydrostreptomycin, have been found to protect liposomes during dehydration. (Emphasis added).

This paragraph says that sucrose interacts with liposome membrane and keeps them intact as the water in the system is removed, and the liposome will survive the dehydration process. In the instant invention, there is no stage of dehydration or rehydration of the liposome; there is only hydration of phospholipids to prepare liposomes. So this quotation is irrelevant. However, upon reading the entire paragraph it is clear that trehalose or sucrose is useful for protection in dehydration, which is not a step in the instant process. The question is -- does Janoff suggest the use of sucrose with ammonium sulphate for hydration of phospholipid to form liposome? No. He does not. Col.8, lines 40 –43 where Janoff describes how vesicles ETV are prepared it is clear that Janoff does not use sugar with ammonium sulphate during hydration of phospholipids for making liposomes. Rather Janoff is using sodium chloride, HEPES buffer with sugar for protecting liposome structure during dehydration. The hydration of phospholipids with ammonium sulphate and sucrose to form liposomes is for the first time shown in the present invention. The question is, is it obvious to one skilled in the art to change from sodium chloride, HEPES and sucrose buffer to ammonium sulphate and sugar as a hydration medium for hydrating phospholipid, and if so for what reason? Kirpotin does not suggest to one skilled in the art to go for ammonium sulphate when it shows that a polymer is much better. Papahadjopoulos does not use ammonium sulphate, so applicants assert that it can not be said

that it is obvious to use ammonium sulphate and sugar as phospholipid hydrating medium for making liposomes.

VIII. Claim Rejections – 35 U.S.C. § 103(a) – Hong/Papahadjopoulos/Janoff/ Radhakrishnan or Uchiyama

In paragraph 9, on page 12[1], claims 1-8, 10, 12, 14-22 and 62 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Hong in view of Papahadjopoulos and Janoff and either Radhakrishnan or Uchiyama. Having regard to the amendment now made to the claims and the comments below, Applicants respectively submit that all claims now in the Application are patentable under 35 U.S.C. § 103(a) and in particular are not obvious over any combination of the teachings of Hong, Papahadjopoulos, Janoff, Radhakrishnan and Uchiyama.

All of the references have been discussed above. When one considers Hong, applicants submit that there is no suggestion, teaching or motivation, to change to solvent removal step from Papahadjopoulos, for getting non-pegylated liposome having more circulation time, when the whole intension of Hong is to study the effect of PEG-DSPE and to try and improve on it by increasing the surface coating of liposomes with PEG. Radhakrishnan and Uchiyama do not teach ammonium sulphate and sucrose in their hydration medium. They do not use the same type of phospholipids. Radhakrishnan has some reference to DSPC and cites a volume of aqueous medium, but eh uses only a phosphate buffered saline. Thus the combination of all these references does not teach or suggest all the elements of the instant invention. Further, none of these references discuss sizing, removing extra liposomal salts, nor are they related to a long-circulation liposome.

In the last paragraph, the Office Action particularly points out the combination of Hong, Papahadjopoulos, Janoff, Radhakrishnan and Emanuel to be considered for claim 62 (now claim 1). This shows that 5 different prior art documents are required for showing obviousness of the instant invention. This by itself shows inventive merit in the instant invention. In spite of referring to these 5 documents and selecting out of context words and lines from those documents, the learned Office Action is unable to synthesize the instant invention.

[1] The Office Action contains two paragraphs numbered as "9."

In addition, Papahadjopoulos and Radhakrishnan are specifically concerned with incorporating the active material during liposome formation whereas Hong, Janoff, and Uchiyama are concerned with loading the active material into a pre-formed liposome and hence, when considering patentability of the amended claims, there is no reason for combining Papahadjopoulos or Radhakrishnan with Hong, Janoff and/or Uchiyama.

The lack of teaching of the combination of Hong, Papahadjopoulos and Janoff is not resolved by either Radhakrishnan or Uchiyama. As mentioned above, neither purports to contribute anything new to the production of liposomes nor mentions either ammonium sulfate or sucrose. The patentability of the amended claims does not depend solely on the amount of aqueous hydrating medium used and accordingly the mere fact that Radhakrishnan (but not Uchiyama) does disclose amounts within and overlapping with that required by claim 1 does not provide the missing information to lead to a process in accordance with amended claim 1.

Further additions of Radhakrishnan and/or Uchiyama are of no use as the secondary and tertiary references failed to show any desired steps which were essential for showing obviousness of the instant invention.

IX. Claim Rejections – 35 U.S.C. § 103(a) – Hong/Papahadjopoulos/Janoff/ Radhakrishnan or Uchiyama/Emanuel

In paragraph 10, on page 13, claim 62 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Hong in view of Papahadjopoulos, Janoff and either Radhakrishnan or Uchiyama, further in view of Emanuel. Having regard to the amendment now made to the claims and the comments below, Applicants respectively submit that all claims now in the Application are patentable under 35 U.S.C. § 103(a) and in particular are not obvious over any combination of the teachings of Hong, Papahadjopoulos, Janoff, Radhakrishnan, Uchiyama, and Emanuel.

Hong, Papahadjopoulos, Janoff, Radhakrishnan, Uchiyama, and Emanuel have all been discussed above. Claim 1, which requires sizing the liposomes to about 0.06 μm and the use of a sucrose-histidine buffer solution to remove extra-liposomal hydration salt, is patentable. Moreover, none of Hong, Papahadjopoulos, Janoff, Radhakrishnan, Uchiyama and Emanuel discloses the use of histidine and sucrose to remove extra-liposomal hydration salt from liposomes. Further, none of Hong, Janoff, Radhakrishnan, and Uchiyama refers to the use of a

buffer containing histidine or sucrose but, as mentioned above, and Papahadjopoulos & Emanuel do disclose use of a histidine buffer. However, the Papahadjopoulos buffers do not contain sucrose, however both the Papahadjopoulos and Emanuel buffers are internal to the liposomes and not used to remove extra-liposomal hydration salt.

As mentioned previously, it is improper, when considering patentability of the amended claims, to combine Papahadjopoulos and Radhakrishnan with Hong, Janoff and/or Uchiyama because Papahadjopoulos and Radhakrishnan are specifically concerned with incorporating the active material during liposome formation whereas Hong, Janoff and Uchiyama are specifically concerned with the loading of active material into formed liposomes.

CONCLUSION

Thus, applicants have shown that none of the cited references actually teach each and every element of the claim nor do they suggest these elements, and the combination of every cited reference does not teach or suggest all of the claim elements, despite the Office Action's attempts to pick and chose elements from each of the references to substitute buffers used for one purpose into buffers used for another purpose. Accordingly, applicants request withdrawal of all of the rejection and request allowance of the present claims. The Commissioner is hereby authorized to charge any fee or credit any overpayment to Deposit Account No: 50-4254.

Respectfully Submitted,

Date: 11/26/08

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